

## **The fine structure of the rat metrial gland in relation to the origin of the granulated cells**

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### **INTRODUCTION**

The fine structure of the rat metrial gland has been the subject of several previous studies (Wislocki, Weiss, Burgos & Ellis, 1957; Larkin & Flickinger, 1969; Dixon & Bulmer, 1971). The distinctive metrial gland cell is characterized by its content of membrane-bound granules, often seen arranged around an extensive Golgi complex. Some granules have a homogeneous dense appearance but many are associated with vesicles or myelin figures. Larkin (1972) has described morphological evidence of granule release in the last week of gestation. The other definitive cell type associated with the metrial gland has been termed a dark cell (Dixon & Bulmer) or stromal cell (Larkin) and resembles a fibroblast.

The origin of the metrial gland cell has been largely a matter of surmise. Both Dixon & Bulmer and Larkin have suggested the precursor cell type may be stromal and Larkin considered that the proliferative activity in the metrial gland was consistent with this view. Smith (1966), in a light microscope study of the mouse uterus in early pregnancy, suggested the possibility that metrial gland cells arose from a white blood cell precursor, but supported this suggestion only by camera lucida drawings.

This paper describes the fine structure of the different cell types occurring in the metrial gland and an attempt is made on the basis of the morphology to follow the differentiation of the granulated cells from their precursors.

### **MATERIALS AND METHODS**

Pregnant Wistar rats were killed on day 12, 14, 16, 18 or 20 of pregnancy. Day 0 was taken as the day on which spermatozoa were detected in the vaginal smear. Metrial gland tissue was taken from anaesthetized animals and fixed in a mixture of 4 % formaldehyde and 2 % glutaraldehyde in 0.1 M phosphate buffer at pH 7.2–7.4. Specimens were washed overnight in buffer and post-fixed in buffered 1 % osmium tetroxide for 1 hour (Palade, 1952). They were then dehydrated in acetone and embedded in Araldite. Silver/gold sections were cut using glass knives on an LKB Ultratome III ultramicrotome. They were mounted on copper grids, stained in 2 % uranyl acetate and lead citrate and examined on a Philips 201 electron microscope.

## OBSERVATIONS

At all stages of pregnancy examined, typical granulated metrial gland cells were seen. The cells varied in size up to about 25  $\mu\text{m}$  in diameter and were usually spherical or oval in shape. The outline of the cells was often irregular, with short projections of the cytoplasmic surface (Figs. 1, 2). The granules, up to 2  $\mu\text{m}$  in diameter, were membrane-bound, pleomorphic structures and usually had a densely stained homogeneous core. In some granules the core was surrounded by a ring of small vesicles while other granules contained myelin figures. The granules were often observed to be arranged in a circle around an area of cytoplasm containing an extensive Golgi network and some oval or spherical mitochondria; in the centre of this area centrioles were sometimes seen. Beyond the granules the peripheral cytoplasm often appeared more lightly stained, but its content of darkly stained particles resulted in a stippled appearance; occasional stacks of rough endoplasmic reticulum occurred, along with a few mitochondria. The outermost rim of cytoplasm was often less stippled and devoid of organelles. Some granulated metrial gland cells were in mitosis; others appeared to be binucleate. The nuclear chromatin was usually uniformly pale-staining, except for a slightly more darkly stained outer rim (Fig. 1). Nucleoli were typically a prominent feature of the granulated metrial gland cell.

The relatively pale-staining granulated metrial gland cell contrasted with a more darkly stained cell type which often appeared similar in shape to a fibroblast. Such dark cells were often closely related to the granulated cells. In some cases this relationship took the form of extensive cytoplasmic processes of the dark cells appearing to surround the granulated cell (Fig. 2). The cytoplasmic extensions of some dark cells were even more marked than those illustrated in Fig. 2, but they did not always appear to be related to granulated cells. The cytoplasm of the darkly stained cells contained numerous oval or elongated mitochondria (some particularly large), extensive endoplasmic reticulum, Golgi bodies, and occasional small dense bodies resembling lysosomes. In some cells a central area of the cytoplasm was devoid of organelles (Fig. 2). In some cells vacuoles were a prominent feature; these often appeared deep within the cell and seemed to contain intercellular matrix. The vacuolar appearance may be a reflexion of the extensive surface folding of the dark cell. The nuclei of the dark cells were often oval-shaped, with much euchromatin and a prominent nucleolus.

The extent and appearance of the intercellular matrix varied in different regions of the same metrial gland and at different stages of pregnancy. Collagen did not appear to be specifically related to either the dark cells or the granulated cells, but was seen in close association with both cell types. In some areas flocculent pale staining material was apparent (Fig. 1), but in other areas little matrix was detected (Fig. 2).

In the last week of pregnancy degenerative changes were apparent in some areas of the metrial gland. Some granulated cells appeared to have undergone lytic changes and cell organelles and granules were seen in the intercellular matrix. These changes were accompanied by the appearance of intracellular lipid, and will be the subject of a further publication.

Several cells were seen which did not fit readily into either of the cell types described above. For instance, this applies to the cells arrowed in Fig. 1. Though some

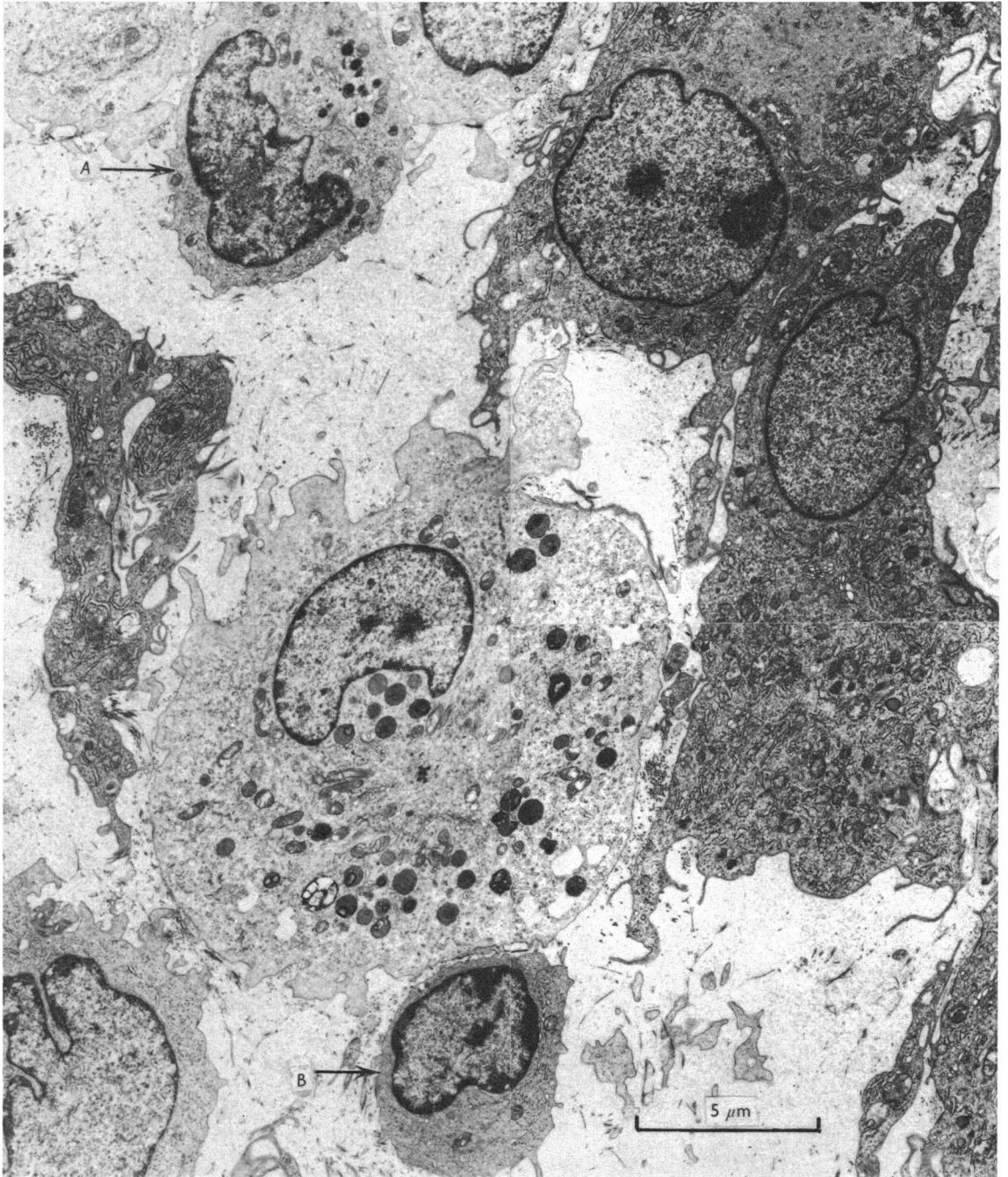


Fig. 1. Metrial gland, day 16 of pregnancy. In the centre of the field there is a granulated cell, with marked surface irregularities. Parts of two dark cells occur to the right of the figure and at the top right an inner area of the cytoplasm is distinguished by relative absence of organelles. The cells arrowed *A* and *B* do not show features of typical dark cells, although the cell *A* has some of the features of a granulated cell.



Fig. 2. Metrial gland, day 18 of pregnancy. The granulated cell (top right) shows a typical close relationship with cytoplasmic extensions from darkly stained cells. The cells arrowed *A* and *B* are readily distinguished from the dark cells, but the cell at *A* may be a granulated cell whose granules were not in the plane of section.

granules can be seen in cell *A* it does not have the appearance of a mature granulated metrial gland cell. In Fig. 2 the cell arrowed at *A* has some of the features of a granulated cell: the apparent absence of granules could be due to the plane of section. The cells arrowed *B* in Figs. 1 and 2, however, do not appear to be related to either dark cells or granulated cells. In Fig. 3 a cell with the pale-staining cytoplasm characteristic of the granulated cells is readily distinguished from the surrounding darkly stained cells, but it cannot be categorized as a granulated cell. Detailed study of the cells which could not readily be categorized suggested that they formed members of a graded series. This is illustrated in Fig. 5, which shows electron micrographs of cells occurring in the metrial gland at the 14 day stage.

Each cell has been placed so that it is minimally different from its neighbours in the series. Though the cell in Fig. 5A has few granules it appears to be otherwise comparable with the typical granulated cell. It is similar in the form of the nucleus; the cytoplasm is lightly stained and stippled, and a few short projections extend from the cell surface. There are fewer granules in the cell illustrated in Fig. 5B, and in the nucleus the nucleolus is not a prominent feature. In Fig. 5C the apparent absence of granules from the cell and of the nucleolus from the nucleus may be due to the plane of section; otherwise there are no features distinguishing it from the cells in Figs. 5B and 5A. Fig. 5D, however, shows a marked change in the nuclear chromatin; there is an increase in the amount of heterochromatin, particularly around the outer rim of the nucleus. The cytoplasm, however, is palely stained, in contrast to surrounding tissue, and apart from the absence of granules it shows no obvious differences from the cells illustrated in Figs. 5A–C. Marked nuclear heterochromatin was not seen in cells larger in size than the one illustrated in Fig. 5D. Figs. 5D–F show cells with varied nuclear/cytoplasmic size ratios. It is likely that the ratio indicated in Fig. 5E is due to sectioning artefact. In both Figs. 5E and 5F the nuclear heterochromatin is pronounced; the cytoplasm is still more palely stained than the surrounding tissue, and cytoplasmic projections are apparent in Fig. 5E. The darkly stained bodies seen in Fig. 5F may be primary lysosomes or developing metrial gland cell granules. As the series progresses through the cells illustrated in Figs. 5G, H and I the heterochromatic nature of the nuclear rim is maintained and cytoplasmic organelles are scarce. In Fig. 5H cytoplasmic projections are a notable feature.

The later cells in the series are shown in Figs. 5K–M and in all of these the nuclear/cytoplasmic size ratio is high. The nuclear heterochromatin is a prominent feature. In Fig. 5M the cytoplasm contains a few organelles, but in Figs. 5L and K organelles are not obvious and the dark stippled appearance of the cytoplasm (a prominent feature of the cells at the beginning of the series) is not present. Cells such as those illustrated in Figs. 5K–M could not be described as palely staining in comparison to adjacent structures. There appeared to be two forms of the final members of the series; the cell in Fig. 5M, surrounded by sparse intercellular matrix, has irregular projections from its surface. In Fig. 5L the more regular cell surface is adjacent to the dense surrounding material. The eccentric nuclear position shown in Fig. 5K was not a common observation.

The morphological gradations which are illustrated and described suggest that reversal of the series shown in Fig. 5 would define the origin of the young



Fig. 3. Metrial gland, day 14 of pregnancy. A pale-staining cell contrasts with the surrounding dark, stromal cells; the absence of granules means that it cannot definitely be termed a granulated cell.



Fig. 4. Metrial gland, day 12 of pregnancy. A blood vessel of the metrial gland contains several mononuclear round cells. Haematoxylin and eosin,  $\times 750$ .

granulated metrial gland cell (Fig. 5A) from a small round darkly staining cell with a markedly heterochromatic nucleus and a high nuclear/cytoplasmic size ratio. Though all the cells shown in Fig. 5 were from the metrial gland at 14 days, similar cell types were also identified at days 12, 16, and 18.

#### DISCUSSION

The fine structure of the granulated metrial gland cell shows that it is a distinctive cell type with numerous densely stained, pleomorphic granules, often appearing in a circular arrangement around Golgi bodies, mitochondria and centrioles. The cytoplasm outside the granules is often fairly pale-staining, has few mitochondria, occasional lengths or stacks of rough endoplasmic reticulum and a finely stippled appearance. The peripheral rim of cytoplasm is often without organelles and the surface of the cells shows irregular projections. As Larkin (1972) pointed out, the pale-staining cytoplasm of the granulated metrial gland cell, contrasting with the more densely stained cytoplasm of the non-granulated cells, allows easy identification even when granules are not present because of the plane of section.

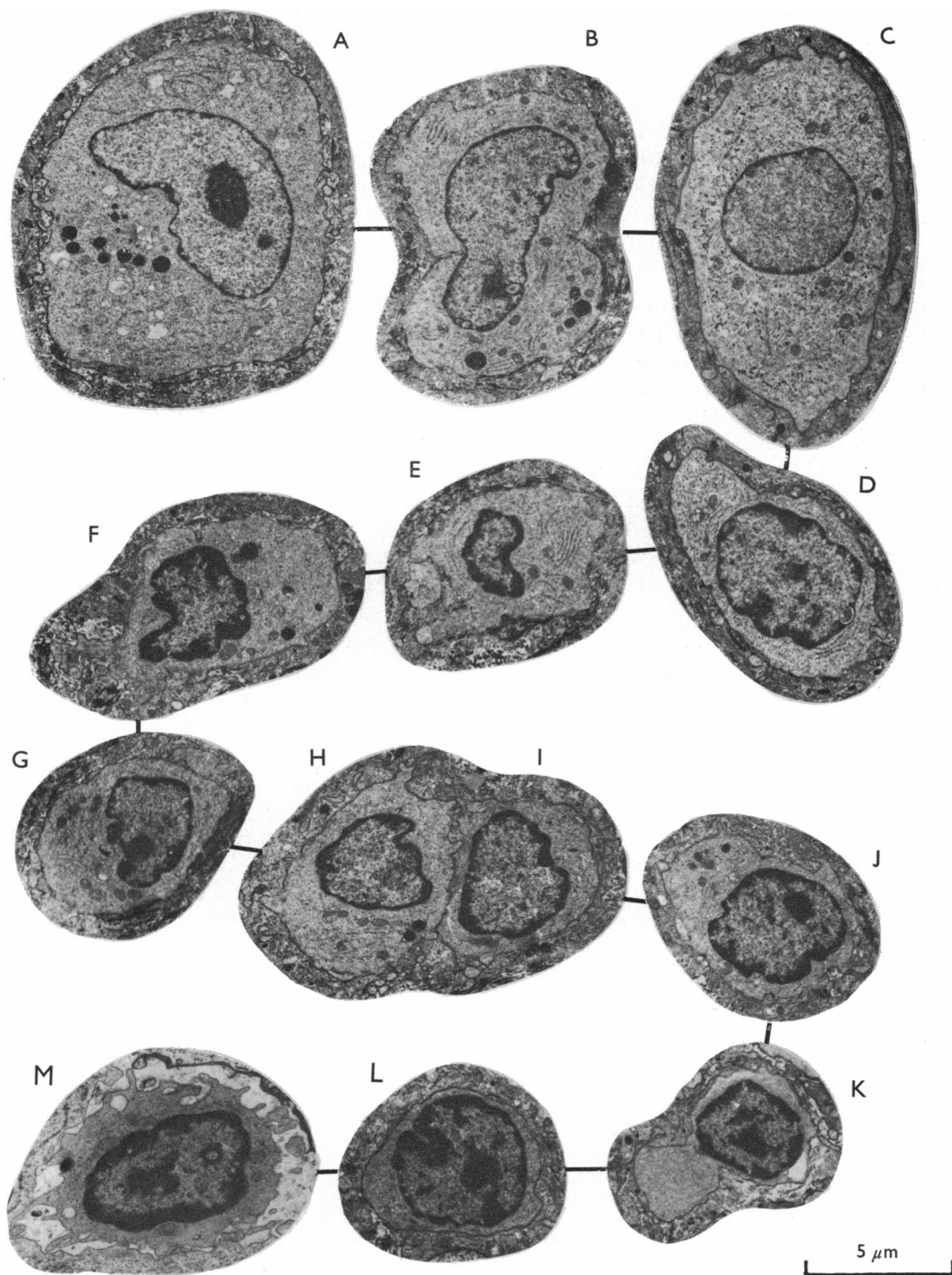


Fig. 5. These illustrations show cells, from the metrial gland at day 14, which could not be categorized as either mature granulated cells or darkly stained stromal cells. They have been arranged in a series so that each cell in the series closely resembles its neighbours.

The darkly staining cells which often appear to surround the granulated metrial gland cell have been described previously and related to similar cells in the decidua basalis (Dixon & Bulmer, 1971). Larkin (1972) suggests that in degenerating regions of the metrial gland after day 15 of pregnancy the only dark cells seen are macrophages. In this study dark cells other than macrophages were seen in some areas of the metrial gland throughout all stages of pregnancy.

It was suggested by Dixon & Bulmer that metrial gland cells may originate from darkly stained cells. However, they pointed out that no intermediate stages were observed and suggested that both metrial gland cells and dark cells may be derived from an undifferentiated connective tissue cell. Previously Larkin & Schultz (1968), in an autoradiographic study at the light microscope level, had suggested fibroblasts in the mesometrial triangle as precursors of the granulated metrial gland cell. In contrast, the observations of Smith (1966) on the differentiation of the metrial gland cell and other glycogen containing cells in the mouse uterus up to day 7 of pregnancy, had led her to suggest that the precursor of metrial gland cells was a cell of the lymphocyte series. Her camera lucida drawings illustrated a continuous series of transitional forms linking the mature metrial gland cell to a small, round basophilic cell which she related to the large lymphocyte.

Detailed examination of the cell types seen in electron micrographs of the metrial gland makes it obvious that certain cells would not fit into categories which have been described previously, that is, granulated cells, dark fibroblast-like cells, lipid-containing cells and macrophages. It was possible to place the uncategorized cell types in a series (Fig. 5) on the basis of their morphological characteristics. It appears possible therefore that the granulated metrial gland cell is related to a small round precursor cell with a high nuclear/cytoplasmic size ratio, dense but uniformly stained cytoplasm with few organelles, and a nucleus with much heterochromatin. From its fine structural appearance this precursor cell would appear to be a small lymphocyte (Zucker-Franklin, 1969). Cells such as that arrowed *B* in Fig. 1 could be described as reactive lymphocytes. The unusual shape of the cell in Fig. 5K could be compared to the hand mirror shape of the lymphocyte as it migrates through tissues (Weiss, 1972).

If differentiation of granulated metrial gland cells *in situ* from lymphocytes does occur (as Smith suggested for the early stages of pregnancy in the mouse), then this ultrastructural study suggests that it must be a process which continues at least up to day 18 of pregnancy, because cells similar to all the stages illustrated in Figure 5 could also be found at days 16 and 18. The origin of the lymphocyte-like cell is not clear, but migration from the blood seems a likely possibility. Bridgman (1948) has remarked on the massive numbers of white cells in blood vessels of the decidua at about day 9 of pregnancy, and it is common to observe disproportionate numbers of white cells (polymorphonuclear leucocytes and mononuclear round cells) in some blood vessels of the metrial gland in the later stages of pregnancy (Fig. 4).

The function of the metrial gland has been a matter of much speculation and no hypothesis has found wide acceptance. The discharge of granules from metrial gland cells into the surrounding stroma in the later stages of pregnancy, and the appearance of metrial gland cells in the blood vessels of the gland throughout pregnancy, have been related to a number of possible functions. Bloch (1964) suggested that metrial

gland cells produced a holocrine secretion involved in disrupting the circular muscle fibres at the base of the mesometrium. Dallenbach-Hellweg, Battista & Dallenbach (1965) suggested that the metrial gland is a source of relaxin secretion, though recent evidence contradicts this view (Larkin, 1974).

The apparent morphological relationship of the granulated metrial gland cell to a small cell resembling a lymphocyte implies that attention should be directed towards the role of the metrial gland in the immunology of pregnancy. The role of the lymphocyte in pregnancy has been the subject of extensive investigation, and it is accepted that changes in lymphocyte distribution and traffic do occur in pregnancy (Howe, 1975).

A role for the metrial gland in the immunological complexities of pregnancy is only hypothetical at this stage. It is perhaps relevant, however, to point out that metrial gland cells, with their peri-arteriolar arrangement and their situation in intercellular spaces, are ideally placed either to migrate to the placenta, or to send their secretions there, possibly in the form of a blocking antibody affecting the trophoblast. Although the metrial gland as an anatomical entity is peculiar to rodents, cell types similar to metrial gland cells have been described in many other animals, including primates (Dallenbach-Hellweg, Dawson & Hisaw, 1966).

#### SUMMARY

The fine structure of the metrial gland of the rat was examined at days 12, 14, 16, 18 and 20 of pregnancy and at all these stages two well differentiated cell types were seen. A relatively pale-staining cell which contained numerous dense granules was readily distinguished from a darkly stained fibroblast-like cell. Numerous cells could be identified, however, which did not fit either of these categories. On the basis of their morphological appearances these cells were placed in a series, with the members of the series showing a transition from a small cell with a high nuclear/cytoplasmic size ratio to a readily identifiable metrial gland cell containing a few typical granules. The small cell, apparently the precursor of the granulated metrial gland cell, was characterized by a markedly heterochromatic nucleus and few cytoplasmic organelles, although there were occasional mitochondria and Golgi bodies. On morphological grounds this cell appeared to be from the lymphocyte series and was probably a small lymphocyte.

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#### REFERENCES

- BLOCH, S. (1964). Die Glandula myometrialis im Uterus der Maus. *Acta anatomica* **56**, 103–119.
- BRIDGMAN, J. (1948). A morphological study of the development of the placenta of the rat. *Journal of Morphology* **83**, 61–85.
- DALLENBACH-HELLWEG, G., BATTISTA, J. V. & DALLENBACH, F. O. (1965). Immunohistological and histochemical localisation of relaxin in the metrial gland of the pregnant rat. *American Journal of Anatomy* **117**, 433–450.
- DALLENBACH-HELLWEG, G., DAWSON, A. B. & HISAW, F. L. (1966). The effect of relaxin on the endometrium of monkeys. Histological and histochemical studies. *American Journal of Anatomy* **119**, 61–78.
- DIXON, A. D. & BULMER, D. (1971). The fine structure of cells in the rat metrial gland. *Journal of Anatomy* **108**, 123–133.

- HOWE, C. (1975). Lymphocyte physiology during pregnancy. In *Immunobiology of the Trophoblast* (ed. R. G. Edwards, C. W. S. Howe and M. H. Johnson), pp. 131–146. Cambridge University Press.
- LARKIN, L. H. (1972). Electron microscopy of granule release in metrial gland cells of the pregnant rat. *Anatomical Record* **172**, 109–126.
- LARKIN, L. H. (1974). Bioassay of metrial gland extracts for relaxin using the mouse interpubic ligament technique. *Endocrinology* **94**, 567–570.
- LARKIN, L. H. & FLICKINGER, C. J. (1969). Ultrastructure of the metrial gland cell in the pregnant rat. *American Journal of Anatomy* **126**, 337–354.
- LARKIN, L. H. & SCHULTZ, R. L. (1968). Histochemical and autoradiographic studies of the formation of the metrial gland in the pregnant rat. *American Journal of Anatomy* **122**, 607–619.
- PALADE, G. E. (1952). A study of fixation for electron microscopy. *Journal of Experimental Medicine* **95**, 285–297.
- SMITH, L. J. (1966). Metrial gland and other glycogen containing cells in the mouse uterus following mating and through implantation of the embryo. *American Journal of Anatomy* **119**, 15–24.
- WEISS, L. (1972). *The Cells and Tissues of the Immune System*, p. 134. New Jersey: Prentice-Hall.
- WISLOCKI, G. B., WEISS, L. P., BURGOS, M. H. & ELLIS, R. A. (1957). The cytology, histochemistry and electron microscopy of the granular cells of the metrial gland of the gravid rat. *Journal of Anatomy* **91**, 130–140.
- ZUCKER-FRANKLIN, D. (1969). The ultrastructure of lymphocytes. *Seminars in Hematology* **6**, 4–27.